Technetium- and Rhenium-Labeled Progestins: Synthesis, Receptor Binding and In Vivo Distribution of an 11β-Substituted Progestin Labeled with Technetium-99 and Rhenium-186

James P. DiZio, Carolyn J. Anderson, Alan Davison, Gary J. Ehrhardt, Kathryn E. Carlson, Michael J. Welch, and John A. Katzenellenbogen

Department of Chemistry, University of Illinois, Urbana, Illinois; Radiation Sciences, Mallinckrodt Institute, Washington University Medical School, St. Louis, Missouri; Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts; and Research Reactor, University of Missouri, Columbia, Missouri

In an effort to develop radiopharmaceuticals useful for the diagnostic imaging of steroid receptor-positive breast tumors, we have radiolabeled an analog of the antiprogestin RU486 (mifepristone), modified to incorporate an N_2S_2 chelate system in the 11β-position, with ^{99}Tc, ^{99m}Tc, and ^{186}Re. For the ^{99}Tc-labeled analogs (**3**), a syn pair and two individual antidiastereomers (linker methylene versus metal-oxo, relative to the N_2S_2 plane) were isolated. In competitive radiometric binding assays, the syn pair (**3syn1,2**) had affinity for the progesterone receptor that was 25% that of (promegestone) R5020 (or 161% that of progesterone), and the individual anti-diastereomers had affinities of 47% (**3anti1**) and 7% (**3anti2**) that of R5020 (or 303% and 45% that of progesterone). The specific-to-nonspecific binding ratio of the ^{99m}Tc (**4**) and ^{186}Re (**5**) 11β-linked syn systems are 75/25 and 54/46, respectively. In vivo, conjugates **4** and **5** showed progesterone receptor-mediated uptake in rat uterus, but also high uptake in non-target tissues, presumably because of the high lipophilicity of the metal complexes. Modified systems may be useful in vivo as receptor-directed agents for diagnostic imaging or treatment of steroid receptor-positive tumors.

J Nucl Med 1992; 33:558–569

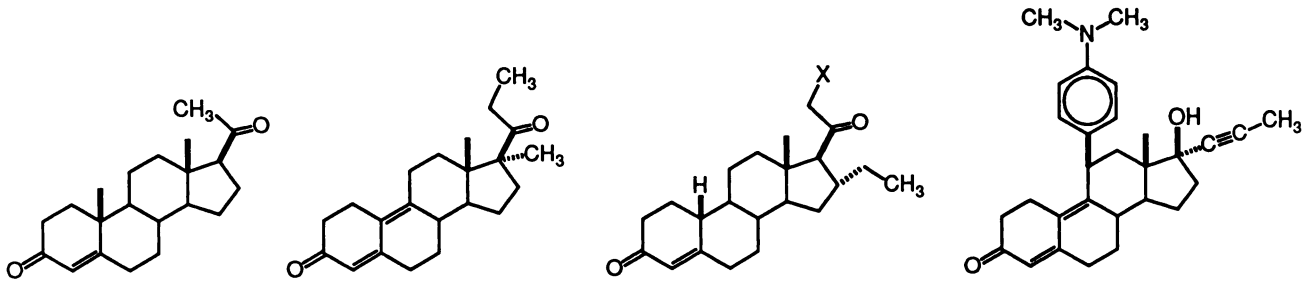
Receptor-binding radiopharmaceuticals are typically receptor ligands labeled by isotopic substitution or by substitution with single atom radionuclides that do not effect a major alteration in the size, physical properties, or pharmacokinetic behavior of the ligand. In fact, we (*1–5*) and others (*6–8*) have used radiohalogen substitution with ^{18}F, ^{77}Br, and ^{123}I as the means for labeling steroids to be used as in vivo imaging agents for hormone receptor-positive tumors. These successes notwithstanding, the difficulties in the production and distribution of these radionuclides have limited the widespread utility of these receptor-binding radiopharmaceuticals. A method by which such imaging agents could be labeled with the readily available ^{99m}Tc in a manner that would preserve favorable receptor binding and imaging characteristics would greatly expand their potential applications.

The challenge in such a strategy is readily apparent, as methods for labeling molecules with metals have required the use of chelating systems (*9,10*) that are bulky and have a mass comparable to that of the receptor ligand itself. Conjugation with such a metal complex may diminish receptor affinity through steric interference with binding and can alter physical properties that affect the adsorption, metabolism, and clearance characteristics of the radiopharmaceutical so that its in vivo distribution is no longer favorable for imaging.

Recently, we undertook a systematic study of progestin-rhenium conjugates in which an N_2S_2 oxorhenium complex was affixed to a ligand for the progesterone receptor at four sites known to be tolerant of bulky substitution (*11*). While three of these conjugates had very low affinity for the progesterone receptor, one, an analog of the Roussel-Uclaf antiprogestin RU38486 (mifepristone, RU486), retained high affinity for the receptor (see Scheme 1 for steroid structures). Of the four oxorhenium complex diastereomers, a syn pair and two individual anti diastereomers were separable, and of these, an anti diastereomer had the highest affinity. In this study, we report the synthesis of these compounds labeled with ^{99}Tc (**3**), ^{99m}Tc (**4**), and ^{186}Re (**5**) and the determination of the binding characteristics of these radiolabeled complexes for the progesterone and other steroid receptors, and the measurement of their distribution in estrogen-primed immature rats in vivo.

Received Aug. 9, 1991; revision accepted Oct. 16, 1991.

For reprints contact: John A. Katzenellenbogen, Department of Chemistry, University of Illinois, 461 Roger Adams Laboratory, Box 37, 1209 West California St., Urbana, IL 61801.



SCHEME 1. Structures of progesterone and synthetic progesterone.

MATERIALS AND METHODS

Biological Procedures

Materials. Radioligands were obtained from the following sources: [1,2,6,7-^{3}H]aldosterone, 81 Ci/mmol (Amersham Corp., Arlington Heights, IL); [17α-methyl-^{3}H]-promegestone (R5020), 86 Ci/mmol, and [6-methyl-^{3}H]11β,17β-dihydroxy-6-methyl-17α-(1-propynyl)-androsta-1,4,6-trien-3-one, (RU28362), 77 Ci/mmol (DuPont New England Nuclear, Boston, MA). Unlabeled ligands: promegestone and RU28362 (DuPont New England Nuclear, Boston, MA); 16α-ethyl-21-hydroxy-19-nor-preg-4-ene-3,20 dione (ORG2058) (Organon Corp., Oss, The Netherlands); RU38486 (Roussel-Uclaf, Romainville, France); hydrocortisone and aldosterone (Sigma Chemical Co., St. Louis, MO). Other chemicals were obtained from the following sources: activated charcoal, Trizma base, 3-[N-morpholino]propane-sulfonic acid (MOPS), n-decylamine (Sigma Chemical Co., St. Louis, MO); dextran grade C (Schwarz/Mann, Orangeburg, NY); dimethylformamide (DMF) (Fisher Scientific, Fairlawn, NJ); 2-mercaptoethanol, (ethylenedinitrilo)tetraacetic acid tetrasodium salt (EDTA) and sodium azide (Eastman Organic Chemicals, Rochester, NY); sodium molybdate and tin (II) chloride (Mallinckrodt Inc., St. Louis, MO); Triton X-114 (Central Solvents and Chemicals Co., Bedford Park, IL); 2,5-diphenyloxazole (PPO) (Research Products International Corp., Mt. Prospect, IL); 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP), 1-octanol (Aldrich Chemical Co., Milwaukee, WI); G-25 Sephadex and Blue Dextran (Pharmacia Fine Chemicals, Piscataway, NJ); Glucoscan kit (DuPont-NEN, N. Billerica, MA); dodecyl sodium sulfate (SDS) (Matheson, Coleman & Bell, Norwood, OH). All in vitro assays were done in the following buffer: 0.01 *M* tris, 0.0015 *M* EDTA, 0.02% NaN_3; 20 m*M* Na molybdate, 20% glycerol, pH 7.4, at room temperature.

Cytosols. Cytosols were prepared and stored as previously reported (*12–15*).

Relative Binding Affinity (RBA). Assays were performed as previously reported (*14*). Several concentrations of unlabeled competitor or buffer, together with 10 n*M* tritiated tracer, were incubated with cytosol at 0°C for 18–24 hr. Unbound ligand was removed with charcoal-dextran. Competitor solutions were prepared in 1:1 DMF:buffer to ensure solubility. Progesterone receptor assays utilized rat uterine cytosol from 3-day estrogen-primed immature rats (~1.5 n*M* receptor plus 1 μM hydrocortisone to block any glucocorticoid receptor) and [^{3}H]R5020 as the tracer. Glucocorticoid receptor assays utilized liver cytosol from 3-day adrenalectomized adult male rats (~1 n*M* type II sites) with [^{3}H] RU28362 as the tracer while the mineralocorticoid receptor assays were performed with kidney cytosol from 3-day adrenalectomized adult male rats (~0.2 n*M* type I sites plus 10^{-6} M RU28362 to block type II sites) and [^{3}H]aldosterone as the tracer.

Radioactivity was determined in a Nuclear Chicago Isocap 300 scintillation counter with adjustable windows, set to exclude 95% of the ^{99}Tc counts from the tritium channel. The 5% spill was subtracted from the tritium counts.

Log P Determinations. The log P values were estimated as previously reported (*2*) from the log k'_w values determined by high-performance liquid chromatography (HPLC) following the recommendations of Minick (*16*).

Direct Binding Assays. Rat uterine cytosol containing 1–4 n*M* progesterone receptor, preincubated with 1 μM hydrocortisone, was incubated with various concentrations of 11β-N_2S_2 ^{99m}Tc or ^{186}Re complex in the absence or presence of a 100-fold excess of unlabeled R5020 (R5020 is used to block the progesterone receptor). Incubations were at 0°C for 1–2 hr. Unbound ligand was generally removed by charcoal-dextran, but also by passage through a column of Sephadex G-25. For all in vitro assays, the ^{99m}Tc complex **(4)** was diluted with ^{99}Tc complex **(3)** to give a specific activity of 90 Ci/mmole.

The charcoal-dextran slurry used to remove unbound ligand was prepared as previously reported (*14*) and used in a ratio of one part charcoal-dextran slurry per ten parts cytosol solution at 0°C. Data were organized and reported according to the Scatchard method (*17*). The Sephadex G-25 was swollen overnight in buffer at room temperature. It was then gravity packed into a 0.5 × 6 cm Pasteur pipette column plugged with glass wool. Samples were added to the column and eluted at 0°C with buffer (12 fractions) followed by buffer containing 1% SDS to elute the free conjugate. The void volume was determined by Blue Dextran and the internal volume by [^{3}H]H_2O.

Animal Uptake Methods. Immature female Sprague-Dawley rats (25 days old, ~50 g except where noted) were injected (IV, lateral tail vein), under methoxyflurane (2,2-dichloro-1,1-difluoroethyl-methylether) anesthesia, with 60 μCi of 11β-N_2S_2 ^{99m}Tc or ^{186}Re complex in a 1/1, physiological saline/ethanol solution. To ascertain whether the uptake was mediated by a high-affinity, limited-capacity system, one set of animals was treated with 18 μg unlabeled ORG2058 (RBA = 170% relative to R5020), coinjected with the radiopharmaceutical. The animals were killed by decapitation at the times indicated, and samples of tissue and blood were weighed. Radioactivity in organ and standard samples

was determined with a Beckman Gamma 8000 automatic well-type gamma counter (Beckman Instruments, Fullerton, CA) (*3,4*).

Metabolism Studies. The radiolabeled progestins were extracted from blood or the homogenized uterus with ethanol (*2, 18*). EtOH (0.5 ml) was added to the tissue and the mixture was subjected to centrifugation. The supernatant was separated from the solid mass. The radioactivity of the supernatant was measured and then a 50-μl aliquot was assayed by TLC (5/1, EtOAc/Hexane) by comparison to an authentic sample of labeled compound. No attempt was made to identify the metabolites.

Chemical Procedures

General. Solvents and reagents were purchased from the following commercial sources: Aldrich, Mallinckrodt, Sigma, Fisher, Baker, Eastman or Alfa and were used as received, unless otherwise noted. Sodium pertechnetate in saline solution was eluted from an $^{99}Mo/^{99m}Tc$ generator purchased from E. I. du Pont de Nemours Co. or Mallinckrodt. Technetium-99m-glucoheptonate kits (Glucoscan kits) were purchased from E. I. du Pont de Nemours Co.

Analytical thin-layer chromatography (TLC) was performed using Merck silica gel F-254 glass-backed plates. Visualization was achieved by phosphomolybdic acid (PMA) or anisaldehyde spray reagents, iodine, or UV illumination. Short silica plugs used Woelm silica gel (0.032–0.064 mm) or Merck silica gel (0.040–0.063 mm). HPLC was performed isocratically with a preparative SiO_2 column (Whatman Partisil M-9, 0.9 cm × 50 cm). The eluate was monitored by UV absorbance at 254 nm and a flow-through sodium iodide scintillation detector, where appropriate. Infrared (IR) spectra are given in cm^{-1} with only diagnostic bands reported. Proton magnetic resonance (^1H NMR) spectra at 300 MHz are reported downfield of a tetramethylsilane internal standard (δ scale). Both low- and high-resolution fast atom bombardment (FAB) mass spectra were obtained employing a dithiothreitol matrix. Elemental analyses were performed by Atlantic Microlabs Inc., Norcross, GA. Pure 11β-BAT estradienes (**1** and **2**) were prepared as previously described (*11*).

11β-N-[α-Oxo(N,N′-bis(2′-methyl-2′-mercaptopropyl)-ethylenediaminato)^{99}technetium(V)-p-toluyl]-17α-propynyl-17β-hydroxy-4,9-estradien-3-one (11β-N_2S_2 ^{99}Tc-Chelate, 3). A solution containing 11β-BAT I estradiene **2** (6 mg, 0.0094 mmol), 50 μl 1 N methanolic NaOAc (0.05 mmol), and 1.5 ml MeOH was slowly added dropwise to a stirring, homogeneous solution of $(Bu)_4N^+[^{99}TcOCl_4]^-$ (*19*) in 1.5 ml MeOH, all at room temperature. The reaction was complete in less than 2 min. The resulting yellow-brown reaction mixture was then evaporated under a stream of nitrogen. To separate the steroidal products from inorganic materials, the residue was dissolved in a small amount of CH_2Cl_2 and passed through a short silica column (eluting with 1/20, iPrOH/CH_2Cl_2). The yellow-brown eluate was then concentrated, and purification by normal-phase HPLC (65/35, hexane/[1/20 iPrOH/CH_2Cl_2]) gave 6.1 mg (86%) of syn (yellow-brown) and anti (bright yellow) diastereomeric pairs as amorphous solids in a 5:1 (Syn:Anti) ratio. The anti diastereomers **3anti1** and **3anti2** were separable by HPLC (t_R 17.5 and 18.7 min, respectively), whereas the Syn diastereomeric pair **3syn1,2** (t_R = 13.8 min) could not be separated into its individual diastereomers. Under these HPLC conditions, the free ligand **2** elutes beyond 21.9 min, so the complexes **3** are in ligand-free form. Analyses are given in the Appendix.

11β-N_2S_2 Syn1,2 ^{99m}Tc-Chelate 4. $Na^{99m}TcO_4$ (~100 mCi) in 2 ml saline solution was added to a Glucoscan kit (200 mg glucoheptonate, 0.06 mg $SnCl_2 \cdot 2H_2O$), swirled, and allowed to stand for 15 min at room temperature. A 100-μl aliquot of this Tc-glucoheptonate mixture (4.5 mCi) was added to a solution of 11β-BAT I estradiene **2** (0.5 mg, 0.8 μmol) in 100 μl MeOH, and the reaction was stirred for 15 min at room temperature. The reaction mixture was diluted with 0.5 ml saline and extracted with CH_2Cl_2 (3 × 1 ml). The combined organic layers were dried by passage through an $MgSO_4$ plug and then evaporated over a stream of nitrogen and redissolved in 1/1 CH_2Cl_2/hexane. HPLC purification [35% (1/20, iPrOH/CH_2Cl_2)/ 65% hexane] provided 1.93 mCi (43%) of 11β-N_2S_2 Syn1,2 ^{99m}Tc-chelate **4** (t_R = 13.8 min). As noted in the preceding preparation, this material was ligand free. The specific activity was estimated to be ~100 Ci/μmol; this is based on a 24-hr period that followed the preceding elution. The $^{99}Mo/^{99m}Tc$ generator was rated at 3.1 Ci of ^{99}Mo at the time of elution, and 2.7 Ci of ^{99m}Tc was eluted.

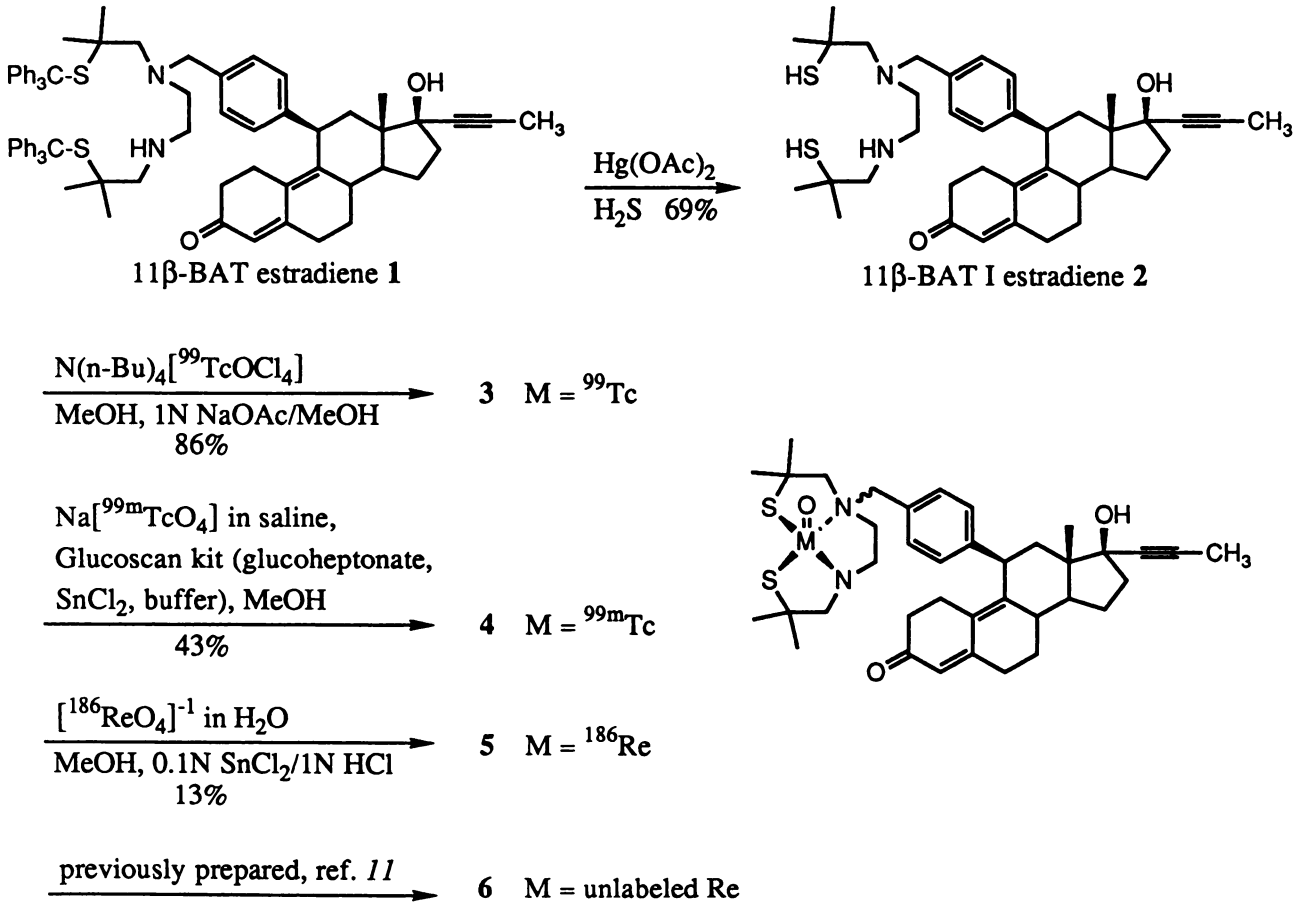
11β-N_2S_2, Syn1,2 ^{186}Re-chelate, 5. Rhenium-186 $(VII)O_4^-$ in H_2O was obtained from the University of Missouri Reactor. The target consisted of 0.25 mg of 95% enriched ^{185}Re as $Al(ReO_4)_3$ and was bombarded for 300 hr with a thermal flux of 3.5×10^{14} neutrons/cm^2/sec. The ^{186}Re produced was dissolved in water and has a specific activity range of 3.5–4.2 Ci/mg Re (651–781 Ci/mmol).

To a solution of 11β-BAT I estradiene **2** (0.5 mg, 0.8 μmol), 0.4 ml EtOH, and 0.15 ml 4N HCl were added to 0.3 ml of $^{186}Re(VII)O_4^-$ (11.35 mCi) in H_2O, with stirring. A portion (100 μl) of a freshly made stock solution of 0.1N $SnCl_2$ (in 1N HCl) was then added, and the reaction was stirred for 45 min at room temperature. The reaction mixture was diluted with 0.5 ml CH_2Cl_2 and neutralized with $NaHCO_3$. The CH_2Cl_2 layer was separated and then the aqueous layer was extracted further with CH_2Cl_2 (2 × 1 ml). The combined organic layers were dried by passage through an $MgSO_4$ plug and then evaporated over a stream of nitrogen. The residue was redissolved in a small amount of CH_2Cl_2 and passed through a small silica column (pipette column, 3 ml EtOAc). The eluent was evaporated under a stream of nitrogen and redissolved in 1/1 CH_2Cl_2/hexane. HPLC purification [40% (1/20, iPrOH/CH_2Cl_2)/60% hexane] provided 2.95 mCi (26%) of 11β-N_2S_2 syn1,2 ^{186}Re chelate **5** (t_R = 12 min); specific activity was 772 Ci/mmol. Trace amounts of the anti diastereomers elute at 18.6 and 20.6 min and free ligand after 22 min. Therefore, the syn diastereomers of **5** were prepared in ligand-free form.

RESULTS

Chemical Synthesis

The synthesis for compounds **3–5** is shown in Scheme 2 and is described below. In each case, the tracer ^{99m}Tc- and ^{186}Re-labeled conjugates **4** and **5** were identified by co-elution upon HPLC analysis with their fully characterized low specific activity or unlabeled counterparts, 11β-N_2S_2 ^{99}Tc-chelate **3** and 11β-N_2S_2 Re-chelate **6** (*11*), respectively. As was discussed in our previous publication (*11*), the insertion of the metal-oxo core into the N_2S_2 ligand can result in the creation of four diastereomeric products for each conjugate. They are denoted as the syn and anti-diastereomeric pairs, syn and anti being defined



SCHEME 2. Synthesis of 11β-BAT 1 estradiene **2** precursor and radioactive chelates **3–5**.

by the orientation of the steroid substituent and the metal-oxo atom relative to the N_2S_2 plane. As has typically been the case (*11,20*), the syn diastereomeric pair was always obtained in higher yield than the anti pair. Because the metal complexes **3–5** elute on normal phase HPLC far ahead of the ligand system 11β-BAT I estradiene (**2**), all of these complexes were prepared in ligand-free form.

11β-BAT I Estradiene 2. S-deprotection of the previously described 11β-BAT estradiene **1** (*11*) was accomplished by treatment with $Hg(OAc)_2$, followed by protonation of the resulting steroidal mercury salt with hydrogen sulfide (*21*). 11β-BAT I estradiene **2** is prone to slow oxidation at ambient conditions ($t_{½}$ = ~4 days), although storage in a closed vial at −20°C can extend the half-life to several months. Impurities due to oxidation do not impede the chelation process. Estradiene **2** was also prepared (*11*) by deprotection of estradiene **1** using $AgNO_3$, but the product was difficult to purify and contained impurities that impeded the subsequent radionuclide chelation reactions.

11β-N_2S_2 ^{99}Tc-Chelate 3. The TcO core was inserted into 11β-BAT I estradiene **2** through ligand exchange with an equivalent quantity of $Bu_4N[^{99}TcOCl_4]$ in a basic methanolic medium (*22*). The syn and anti-diastereomeric pairs were separated by column chromatography. The anti pair could be further separated into its individual diastereomers, **3anti1** and **3anti2** (each bright yellow), by HPLC, whereas the syn pair, **3syn1,2** (yellow-brown), were not separable. The syn-to-anti product ratio was dependent on concentration, the more concentrated the reaction mixture, the less anti product formed. An 8:1 or 30:1, syn:anti ratio of diastereomers was obtained at a 1×10^{-3} *M* or 5×10^{-2} *M* concentration of the steroid and ^{99}Tc reagent, respectively. This type of product ratio versus concentration dependence has been noted by Lever et al. (*20*). Ligand exchange was also performed using Na-[^{99}TcO(OCH$_2$CH$_2$O)$_2$] (*20*), but since the syn-to-anti ratio was 15:1 at 10^{-3} *M*, this method was not further pursued. As amorphous solids, the ^{99}Tc complexes were very stable at ambient conditions. In solution, they were observed to be stable in reagent grade CH_2Cl_2 and MeOH, but unstable in EtOAc and $CDCl_3$.

The IR metal-oxo bond stretch (in cm^{-1}) for the ^{99}Tc and the analogous unlabeled rhenium conjugates are seen below:

	IR metal-oxo bond stretch (cm^{-1})		
	Syn1,2	Anti1	Anti2
Unlabeled rhenium conjugate **6**	941	953	951
^{99}Tc conjugate **3**	920	930	928

As previously observed (*11*), the metal-oxo bond stretch of the syn diastereomer is consistently lower than that of the anti. Also, the TcO bond stretch is about 20 cm^{-1} lower than the corresponding ReO bond stretch.

11β-N_2S_2 Syn1,2 ^{99m}Tc-Chelate 4. Technetium-99m-conjugate **4** was prepared in a chelation exchange reaction with preformed ^{99m}Tc-glucoheptonate and 11β-BAT I estradiene **2** and purified by HPLC. As the anti diastereomers were not formed in appreciable yield at the tracer level, only the syn diastereomeric pair (inseparable from each other, 43% yield) were isolated. The 11β-N_2S_2 ^{99m}Tc conjugate was chemically stable in EtOH for days. The material used for the in vitro and in vivo studies had a radiochemical purity of >95%. The specific activity for the no-carrier-added material was estimated to be ~100,000 Ci/mmol (see Materials and Methods); it was diluted to 90 Ci/mmol with the ^{99}Tc chelate **3** prior to all in vitro binding experiments.

11β-N_2S_2 Syn1,2 ^{186}Re-Chelate 5. Rhenium-186-conjugate **5** was prepared through in situ reduction (using acidic $SnCl_2$) (*23*) of $^{186}ReO_4^-$ in the presence of 11β-BAT I estradiene **2**, and purified by HPLC. As with the ^{99m}Tc analog, only the syn diastereomeric pair (inseparable from each other, 26% yield) was isolated. With rhenium, the reaction times were longer and the yields were lower than with the ^{99m}Tc analogs. The ^{186}Re conjugate was moderately unstable to air oxidation, but was stable for days in a capped vial with EtOH. The complex used for the in vitro and in vivo studies had a radiochemical purity of >95% and a specific activity of ~772 Ci/mmol (see Materials and Methods).

In Vitro Studies

Relative Binding Affinity of ^{99}Tc-Conjugates (3) to the Progesterone Receptor and Other Steroid Receptors. The relative binding affinities (RBA) of the 11β-N_2S_2 ^{99}Tc-conjugates **3**, the analogous, previously prepared (*11*) unlabeled rhenium complexes (**6**), and some related compounds (cf. Scheme 1) are shown in Table 1. The RBAs are determined by a competitive radiometric binding assay. Even though they are radioactive, the ^{99}Tc complexes can be assayed using standard dual-label counting methods in a competitive binding assay with a tritium-labeled tracer. The specific activity (SA) of ^{99}Tc is low (0.0017 Ci/mmol) compared to the SA of tritiated tracer ligands (77–86 Ci/mmol), so the counts from the ^{99}Tc complexes are appreciable only at the highest concentrations.

The RBA values of the ^{99}Tc conjugates **3** for the progesterone receptor are similar and show the same trends as those of the analogous unlabeled rhenium conjugates. This result was expected since the rhenium and technetium chelates are very much the same in terms of size and

TABLE 1
Binding Affinity of the Progestin Metal Complexes and Related Ligands for the Progesterone Receptor (PR), Glucocorticoid Receptor (GR), and Mineralocorticoid Receptor (MR), and Their Log P Values

Compound	RBA* (%) PR† (R5020 = 100)	GR‡ (RU28362 = 100)	MR§ (Aldosterone = 100)	Log P (from k'_w)
Progesterone	15 ± 1	0.56 ± 0.17	not assayed	3.87
RU 486	170 ± 5	60¶	0.02	4.69
ORG 2058	198 ± 39	1.03 ± 0.22	0.92 ± 0.16	4.21
R5020	100	1.46 ± 0.83	0.93 ± 0.05	4.34
11β-BAT I estradiene **2**	11 ± 1	25 ± 10	0.08 ± 0.02	not assayed
FENP	697 ± 88**	0.74 ± 0.24	3.90	4.41
11β-N_2S_2 ^{99}Tc-chelate **3**				
Syn1,2	25 ± 1	29 ± 8	<0.01	6.41
Anti1	47 ± 1	14 ± 4	<0.01	6.25
Anti2	7 ± 1	26 ± 4	<0.01	6.33
11β-N_2S_2 Re-chelate **6**				
Syn1,2	25 ± 4††	16 ± 3	<0.01	6.45
Anti1	44 ± 12††	31 ± 3	<0.01	6.30
Anti2	10 ± 3††	18 ± 7	0.01	6.26

* The receptor binding affinity is determined by a competitive radiometric binding assay. Values are the average of two or more determinations ± range (n = 2) or s.d. (n ≥ 3), and are expressed on a percent scale relative to the affinity of the tritium-labeled tracer (*12*).

† Cytosol preparations were from estrogen primed immature rat uterus, with [^{3}H]R5020 as tracer (*12*).

‡ Cytosol preparations were from saline perfused liver of 3-day adrenalectomized mature male rats with [^{3}H]RU28362 as tracer (*13*).

§ Cytosol preparations were from saline perfused rat kidney with [^{3}H]aldosterone as tracer in the presence of RU28362 to block glucocorticoid receptors (*13*).

¶ Data from ref. 24.

** Data from ref. 25.

†† Data are from ref. 11.

electron density distribution (*24–27*). The ^{99}Tc syn pair (**3syn1,2**) and anti 1 (**3anti1**) have binding affinities that are 1.6 and 3 times that of progesterone, respectively.

Immature rat uterus contains, in addition to progesterone receptor, a substantial amount of glucocorticoid receptor and a small amount of mineralocorticoid receptor (72% and 5%, relative to progesterone receptor = 100%) (Carlson KE, unpublished data). All in vitro studies with the progesterone receptor include a 1-μM concentration of hydrocortisone to block these receptors, but they are not blocked in the in vivo experiments. Most progestins show heterologous binding to these receptors, so that the influence of this binding on the results of in vivo experiments must be considered. As shown in Table 1, the ^{99}Tc and ^{186}Re chelates have affinities for the glucocorticoid receptor nearly equal to their affinities for the progesterone receptor. These glucocorticoid receptor affinities are ~10 times those of the selective progesterone receptor ligands ORG2058 and R5020, but lower than the parent ligand, RU486. The chelates and RU486 all show very low affinities for the mineralocorticoid receptor.

Octanol/water partition coefficients, as a measure of lipophilicity, have been shown to correlate well with the nonspecific binding of steroids (*28*). Recently, the chromatographic method of Minick (*16*) to measure log k'_w has been found to accurately reproduce octanol/water partition values measured with the shake flask method. The regression line relating log k'_w values of the standards to their measured log P values shows very high statistical reliability (*2*) and was extended to include the high log k'_w values of the progestin-chelates, although no standards existed in this range. As shown in Table 1, all of the metal chelates show very high log P values that average about 6.3, which is ca. 100-fold higher than that of the simple steroidal progestins. Thus, in terms of this measure, the metal chelates are extremely lipophilic. This lipophilicity results in increased nonspecific binding and may lead to increased uptake into non-target tissues (*28*).

*Direct Progesterone Receptor Binding Studies of ^{99m}Tc- and ^{186}Re-Conjugates **4** and **5**.* The binding of ^{99m}Tc and ^{186}Re conjugates was measured both by single-point assays, in which the bound and free components were separated by passage through a Sephadex G-25 column, and by multiple concentration binding assays, in which separation was performed by a charcoal-dextran adsorption method.

Data for the single point assays are shown in Figure 1, Panel A (^{99m}Tc conjugate) and Panel B (^{186}Re conjugate) as elution profiles from Sephadex G-25 size exclusion columns. This method of assaying protein binding is robust, since it separates protein bound and unbound conjugate from radioactive impurities such as ReO_4^-, TcO_4^-, and TcO_2. (These inorganic salts are not removed by charcoal, so in that assay they raise the background levels and introduce uncertainty in the interpretation of specific to nonspecific binding ratios.) The conjugate bound to protein eluted in the void volume (fractions 3 and 4) of

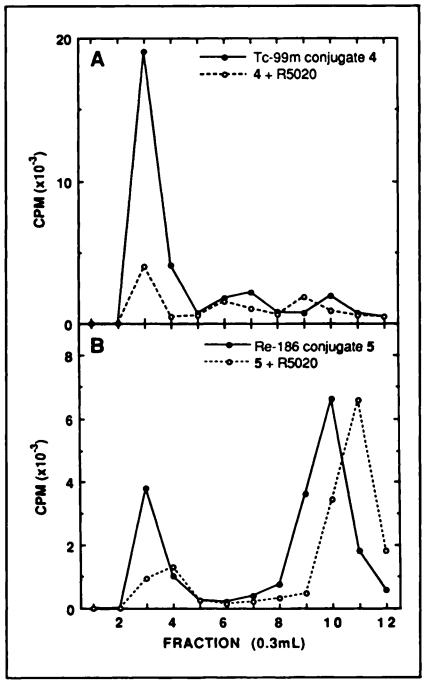


FIGURE 1. Sephadex G-25 elution profile of ^{99m}Tc conjugate **4** (A) and ^{186}Re conjugate **5** (B) after 1 hr incubation in rat uterine cytosol. The solid line represents samples where the conjugate was permitted to bind to total protein. The dashed line represents samples in which progesterone receptor sites were filled with R5020 (blocked). Total volume of each fraction is 0.3 ml. Fractions 3 and 4 represent the column void volume. Unbound, intact conjugate is adsorbed onto the column and eluted by a buffer change to 1% SDS after fraction 12 (data not shown).

the G-25 column, while the later fractions (9–12) contain the metal oxides ReO_4^-, TcO_4^- and TcO_2. With buffer elution, the unbound conjugate is adsorbed on the G-25 column; it can be eluted with 1% sodium dodecyl sulfate (SDS). (As this elution was done after fraction 12, it is not shown in Fig. 1). In each case, the solid line represents the assay of total binding (specific plus nonspecific) with the radiolabeled conjugate alone, and the dashed line represents an assay of nonspecific binding run in parallel, in which binding to the progesterone receptor was blocked with a 100-fold excess of the potent progestin R5020. By this method, which separates the bound fraction from metal oxides, the ratio of specific-to-nonspecific binding for the ^{99m}Tc-conjugate (**4**) is found to be 75/25, and for the ^{186}Re- (**5**) conjugate 54/46. This amount of nonspecific binding is relatively high, since most high affinity, selective ligands for the progesterone receptor have less than 10% nonspecific binding. It probably reflects both the large size and high lipophilicity of these metal complexes (see Table 1 and previous discussion).

The ^{186}Re conjugate shows some decomposition product (fractions 9–12), which is probably the ReO_4^- salt. This decomposition product is seen whether the ^{186}Re conjugate is incubated in buffer alone or in the uterine cytosol. We estimate that the rhenium conjugate decomposes at a rate of about 6% per hour in rat uterine cytosol (by TLC measurement), but can decompose faster, depending on

conditions. Although it appears on the G-25 column as a substantial peak, the metal oxide represents only 7% of the radioactivity in the binding assay. The ^{99m}Tc conjugate appears to be more stable both in buffer and cytosol, and there is little evidence of a decomposition product. [Rhenium complexes are known to be more sensitive to oxidation than technetium complexes (*27*)].

Data for the multipoint binding assays are arranged in Figures 2 (^{99m}Tc) and 3 (^{186}Re). Although the assays are not strictly comparable, these ratios are close to those obtained from the Sephadex G-25 assay. The somewhat higher nonspecific binding for the ^{186}Re-complex **5** in this assay could be due to partial decomposition of the conjugate and the subsequent formation of a product that is not adsorbed by the charcoal-dextran (e.g., ReO_4^-), and thus appears in the fraction in this assay as nonspecific. In both assays, R5020 binds to the progesterone receptor with >90% specificity.

The Scatchard plots in Figures 2C and 3C show the site concentration and high affinity binding of the metal complexes and [^{3}H]R5020. In each case, the metal conjugates bind to nearly the same number of sites as does R5020, consistent with the metal conjugates binding only to the progesterone receptor. The small differences observed in site concentration could easily be due to uncertainties in the estimated specific activities of the radioactive metals. The K_d for [^{3}H]R5020 in those assays is 0.90–1.36 n*M*;

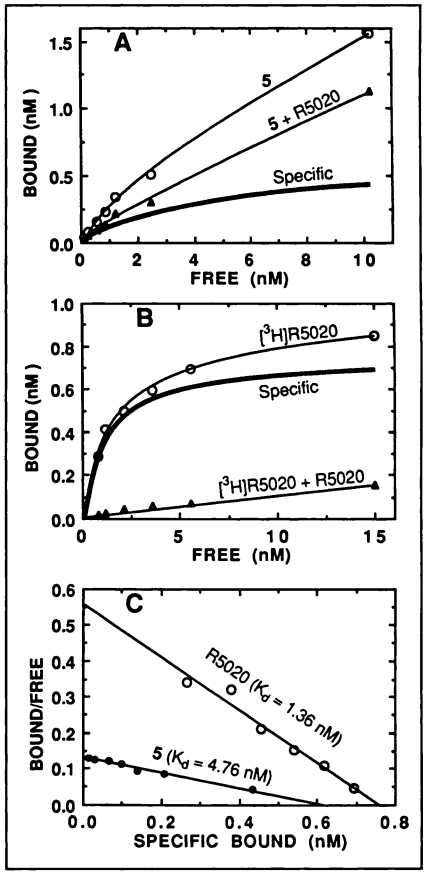


FIGURE 3. Binding curves for ^{186}Re-conjugate **5** (A) and R5020 (B) in rat uterine cytosol. (C) Specific binding data as a Scatchard plot.

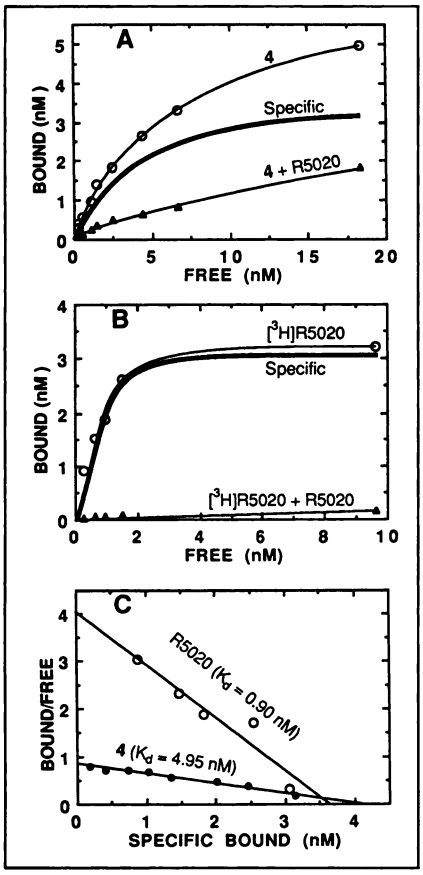


FIGURE 2. Binding curves for ^{99m}Tc conjugate **4** (A) and R5020 (B) in rat uterine cytosol, in which specific: nonspecific binding is greater for ^{99m}Tc-(**4**). (C) Specific binding data for [^{3}H]R5020 complexes **4** and **5** as a Scatchard plot.

the K_d for ^{99m}Tc-complex **4** is 4.95 n*M*, and for the ^{186}Re-complex (**5**), 4.76 n*M*. The ratio of the dissociation constants for the metal conjugate versus R5020 ($K_{d(R5020)}/K_{d(conjugate)}$) is analogous to an RBA value. For the ^{99m}Tc and ^{186}Re conjugates, these ratios are 18% and 29%, very close to the RBA values of 25% and 28%, respectively (Table 1). The concordance of K_d ratios with RBA values is particularly significant, as it verifies that the RBA values obtained in the competitive binding assay (with ^{99}Tc and unlabeled rhenium) are those of the intact metal complexes and not the decomplexed steroid. Both the single-point Sephadex G-25 assays and the multipoint Scatchard assays show that the conjugate is binding to the progesterone receptor with the metal-oxo core intact.

In Vivo Tissue Distribution of ^{186}Re and ^{99m}Tc Conjugates 4 and 5

Tissue uptake studies were performed in immature female Sprague-Dawley rats 21–25 days old that were primed with estradiol to increase their content of uterine progesterone receptor. The rats were injected intravenously with either the ^{99m}Tc (**4**) or the ^{186}Re (**5**) conjugate, and the radioactivity in various tissues was determined after 1, 6, and 18 hr. Some rats were coinjected with a large dose (18 μg) of ORG2058 (RBA = 170% relative to R5020) to block the progesterone receptor sites. ORG2058 is one of the most selective ligands for the progesterone receptor; therefore, it should not block other receptors

TABLE 2
Biodistribution Data for 11β-N_2S_2 ^{99m}Tc-Chelate **4** at 1, 6, and 18 Hours

	%ID/g ± s.d. (n = 5)			%ID/g ± s.d. (n = 5*)	
	1 hr	6 hr	18 hr	6 hr	6 hr (block)
Uterus	0.9856 ± 0.1438	0.6844 ± 0.1458	0.2032 ± 0.0495	0.4879 ± 0.0875	0.2537 ± 0.0700
Blood	0.2250 ± 0.0579	0.1268 ± 0.0160	0.0476 ± 0.0127	0.1076 ± 0.0173	0.1184 ± 0.0187
Fat	0.8912 ± 0.2357	1.9772 ± 0.3665	0.5670 ± 0.0968	1.1117 ± 0.1724	1.4503 ± 0.2287
Muscle	0.7716 ± 0.2446	0.2381 ± 0.0325	0.0558 ± 0.0181	0.1729 ± 0.1171	0.1255 ± 0.0252
Lung	1.6280 ± 0.4780	0.5943 ± 0.1214	0.2218 ± 0.0814	0.3020 ± 0.0609	0.3675 ± 0.0450
Liver	6.7428 ± 1.4661	3.2009 ± 0.5103	1.2254 ± 0.3273	1.3817 ± 0.6864	1.9078 ± 0.3056
Kidney	3.1222 ± 0.7982	1.7260 ± 0.1871	0.8109 ± 0.1459	1.2538 ± 0.1313	1.0952 ± 0.1680
Brain	0.0550 ± 0.0167	0.0269 ± 0.0061	0.0128 ± 0.0044	0.0127 ± 0.0027	0.0155 ± 0.0035
Bone	0.7646 ± 0.2286	0.3271 ± 0.0707	0.0706 ± 0.0278	0.1687 ± 0.0248	0.1609 ± 0.0214
Ovaries	1.0981 ± 0.1953	1.2956 ± 0.1372	0.2549 ± 0.0670	0.7489 ± 0.1426	0.7944 ± 0.2380
Uterus/muscle	1.3205 ± 0.1755	2.8864 ± 0.5421	3.7556 ± 0.4997	3.6283 ± 1.2097	2.0906 ± 0.5898
Uterus/blood	4.4690 ± 0.4384	5.3905 ± 0.7814	4.3157 ± 0.4457	4.5351 ± 0.4186	2.1829 ± 0.5799

The last two columns at 6 hr are for a set of rats that were coinjected with 18 μg of ORG2058 (block) and a control group that were not given the block.

* The rats used in these two experiments (6 hr and 6 hr (Block) were heavier (66.1 ± 6.7 g, n = 16) than those used in the three experiments (first three columns of Table 2; 51.0 ± 9.8 g, n = 15). This difference in weight and possible differences in age-related metabolic rates can account for the somewhat lower uptake seen at 6 hr (column 4) vs. 6 hr (column 2).

(glucocorticoid, mineralocorticoid) present in rat tissues (*13,29*). The tissue uptake data are presented as percent of injected dose per gram tissue (%ID/g) in Tables 2 and 3 and as a percent of the injected dose per organ (%ID/organ) in Tables 4 and 5. Bar graphs comparing the tissue uptake for ^{99m}Tc-conjugate **4** and ^{186}Re-conjugate **5** are shown in Figures 4 and 5 as %ID/g.

The target organ for these radiolabeled progestins is the uterus, and there is substantial uterine uptake for both conjugates. The uterine uptake of the ^{99m}Tc complex is 1.7-fold greater than that of the ^{186}Re complex, 0.986 and 0.593 %ID/g, respectively. It is also retained somewhat longer in the uterus than is the ^{186}Re complex. By 6 hr, 70% of the initial ^{99m}Tc uptake remains, but only 48% of the ^{186}Re uptake. By 18 hr, both complexes have only ~20% of the initial uptake remaining. There is also high uptake in the non-target organs involved in the metabolism and excretion of steroids, liver and kidney, and also in fat. The high fat uptake suggests that the nonspecific uptake may be dominated by the high lipophilicity of these metal complexes; however, binding to the glucocorticoid receptor may also account for some of the uptake into liver and kidney, organs rich in this receptor (*30–32*). Nevertheless, the uptake in the uterus is higher than that in a non-target tissue such as muscle, and the uterus-to-blood ratio after 6 and 18 hr is 4 to 5. Although the uptake

TABLE 3
Biodistribution Data for 11β-N_2S_2 ^{186}Re-chelate **5** at 1, 6, and 18 Hours

	%ID/g ± s.d. (n = 5)			
	1 hr	6 hr	6 hr (block)	18 hr
Uterus	0.5929 ± 0.1998	0.2853 ± 0.0602	0.1418 ± 0.0366	0.1189 ± 0.0249
Blood	0.1766 ± 0.0360	0.0799 ± 0.0073	0.0870 ± 0.0123	0.0220 ± 0.0015
Fat	0.7667 ± 0.4299	0.6621 ± 0.0496	0.7846 ± 0.2168	0.2544 ± 0.0423
Muscle	0.3836 ± 0.1347	0.0935 ± 0.0102	0.0963 ± 0.0296	0.0195 ± 0.0020
Lung	0.8537 ± 0.3288	0.2245 ± 0.0286	0.1895 ± 0.0567	0.0756 ± 0.0146
Liver	2.8239 ± 0.7881	0.8925 ± 0.1948	0.9351 ± 0.1753	0.3347 ± 0.0518
Kidney	1.7311 ± 0.6561	0.4461 ± 0.0271	0.4125 ± 0.0452	0.1848 ± 0.0193
Brain	0.0240 ± 0.0105	0.0100 ± 0.0016	0.0102 ± 0.0021	0.0047 ± 0.0009
Bone	0.4032 ± 0.1293	0.1008 ± 0.0133	0.1067 ± 0.0297	0.0250 ± 0.0051
Ovaries	0.7411 ± 0.3850	0.5024 ± 0.0415	0.4363 ± 0.0556	0.1160 ± 0.0337
Uterus/Muscle	1.5598 ± 0.1682	3.0841 ± 0.6558	1.5344 ± 0.3386	6.1146 ± 1.1173
Uterus/Blood	3.2816 ± 0.4860	3.6418 ± 0.4510	1.6139 ± 0.1797	5.4916 ± 1.3244

One set of animals at 6 hr were coinjected with 18 μg ORG2058 (Block).

TABLE 4
Biodistribution Data for 11β-N_2S_2 ^{99m}Tc-chelate **4** at 1, 6, and 18 Hours

	%ID/Organ ± s.d. (n = 5)			%ID/Organ ± s.d. (n = 5)	
	1 hr	6 hr	18 hr	6 hr	6 hr (block)
Uterus	0.1538 ± 0.0496	0.1006 ± 0.0299	0.0257 ± 0.0054	0.0657 ± 0.0172	0.0303 ± 0.0108
Blood	0.8663 ± 0.1151	0.3408 ± 0.1294	0.1757 ± 0.0357	0.5160 ± 0.0530	0.4970 ± 0.0810
Fat	6.7439 ± 0.9229	10.929 ± 5.5707	4.2530 ± 1.0667	10.528 ± 1.1222	12.031 ± 2.1574
Muscle	5.9546 ± 1.3294	1.2851 ± 0.5478	0.4107 ± 0.1107	1.6179 ± 1.0050	1.0524 ± 0.2081
Lung	0.9436 ± 0.2403	0.2987 ± 0.0986	0.1145 ± 0.0354	0.1801 ± 0.0219	0.2185 ± 0.0296
Liver	16.973 ± 2.0401	6.2653 ± 0.7515	3.7442 ± 0.3677	5.6510 ± 0.4857	5.3499 ± 0.7310
Kidney	1.0539 ± 0.2401	0.5283 ± 0.0588	0.2717 ± 0.0200	0.4930 ± 0.0200	0.3932 ± 0.0532
Rest of Brain	0.0759 ± 0.0201	0.0354 ± 0.0102	0.0170 ± 0.0055	0.0177 ± 0.0041	0.0215 ± 0.0052
Bone	4.5979 ± 0.9458	1.332 ± 0.4730	0.4029 ± 0.1408	1.2613 ± 0.1272	1.0509 ± 0.1373
Ovaries	0.0682 ± 0.0043	0.0657 ± 0.0123	0.0111 ± 0.0028	0.0404 ± 0.0095	0.0516 ± 0.0182

The last two columns at 6 hr are for a set of rats that were coinjected with 18 μg of ORG2058 (block) and a control group that were not given the block.

* The rats used in these two experiments (6 hr and 6 hr (block) were heavier (66.1 ± 6.7 g, n = 16) than those used in the three experiments (first three columns of Table 2; 51.0 ± 9.8 g, n = 15). This difference in weight and possible differences in age-related metabolic rates can account for the somewhat lower uptake seen at 6 hr (column 4) vs. 6 hr (column 2).

into the uterus is highest at 1 hr and drops by ~80% in 18 hr, the contrast between target and non-target (uterus/muscle) continues to improve throughout the 18 hr.

The uterus-to-muscle ratio is slightly higher for the ^{186}Re complex than for the ^{99m}Tc complex at 1 and 6 hr, and substantially better at 18 hr. In contrast, the uterus/blood ratio is higher for the ^{99m}Tc complex at the earlier times. Both ratios measure target-to-non-target uptake. The lower uterus/blood ratio for the ^{186}Re complex probably indicates that there are circulating ^{186}Re metabolites in the blood which are not taken up or retained by the muscle or uterus. The ^{186}Re complex was less stable both in blood and uterus than the ^{99m}Tc complex (see Metabolism Studies below).

When the progesterone receptor is blocked by treatment with a large dose of ORG2058, only the uterus shows a significant decrease in uptake (ca. 50%), suggesting that the uterine uptake is progesterone-receptor mediated. In this blocking experiment, the statistical significance of the changes in the various tissues was calculated by the T-test (*33*), n = 5, and only the change in uterine activity is statistically significant at the 99% confidence level ($0.01>p>0.001$). Thus, it appears that we are observing progesterone receptor-mediated uptake of conjugates **4** and **5** in the uterus. ORG2058 blocks progesterone receptor, but is unlikely to block glucocorticoid receptor. Thus, the uterine uptake of the conjugates which cannot be blocked by ORG2058 may be due to their binding to the glucocorticoid receptor, since both conjugates have substantial affinity for this receptor.

Metabolism Studies

The radioactivity in the blood at various times (up to an hour) after injection was extracted into EtOH and analyzed by TLC (Fig. 6). In most cases, the radioactive species were extracted with >70% efficiency. The percent

TABLE 5
Biodistribution Data for 11β-N_2S_2 ^{186}Re-chelate **5** at 1, 6, and 18 Hours

	%ID/Organ ± s.d. (n = 5)			
	1 hr	6 hr	6 hr (block)	18 hr
Uterus	0.0843 ± 0.0349	0.0443 ± 0.0205	0.0213 ± 0.0033	0.0124 ± 0.0018
Blood	0.7629 ± 0.1403	0.3259 ± 0.0507	0.3396 ± 0.0541	0.0944 ± 0.0119
Fat	6.4909 ± 3.5858	5.4340 ± 0.5519	5.9620 ± 1.2639	2.1538 ± 0.4156
Muscle	3.3031 ± 1.0693	0.7792 ± 0.1044	0.7474 ± 0.2269	0.1668 ± 0.0203
Lung	0.4796 ± 0.1919	0.1275 ± 0.0202	0.1043 ± 0.0319	0.0371 ± 0.0050
Liver	8.3496 ± 2.0855	2.4275 ± 0.5484	2.3460 ± 0.0939	1.0187 ± 0.1208
Kidney	0.6428 ± 0.2147	0.1753 ± 0.0143	0.1448 ± 0.0252	0.0733 ± 0.0041
Rest of Brain	0.0342 ± 0.0144	0.0128 ± 0.0018	0.0129 ± 0.0026	0.0062 ± 0.0012
Bone	2.7092 ± 0.8307	0.6567 ± 0.1191	0.6473 ± 0.1861	0.1677 ± 0.0400
Ovaries	0.0432 ± 0.0265	0.0309 ± 0.0125	0.0266 ± 0.0062	0.0069 ± 0.0038

One set of animals at 6 hr were coinjected with 18 μg ORG2058 (block).

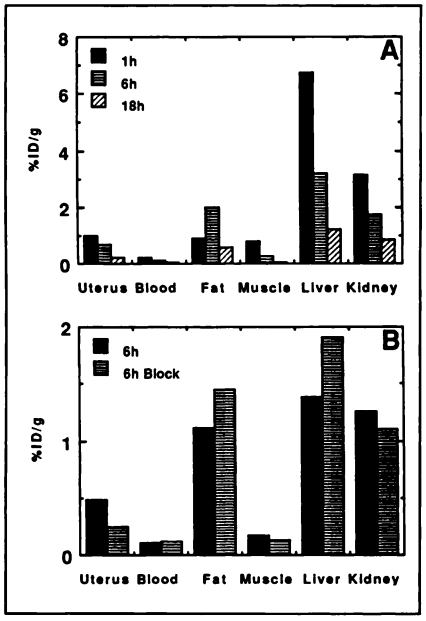


FIGURE 4. Comparison of the uptake in the ^{99m}Tc conjugate **4** in various rat tissues. (A) shows the uptake in various tissues at 1, 6, and 18 hr. (B) Uptake after 6 hr for rats coinjected with 18 μg ORG2058 to block the progesterone receptor and rats not coinjected.

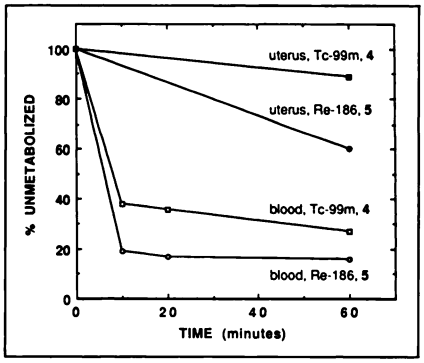


FIGURE 6. Metabolism of ^{99m}Tc and ^{186}Re conjugates **4** and **5** in uterus and blood. In each case, the radiochemical purity (% unmetabolized) is determined by TLC analysis of an EtOH extract of the tissue (*2,18*).

of unmetabolized ^{99m}Tc-conjugate **4** in the blood after an hour was about 25%, and for ^{186}Re-conjugate **5**, 15%. This means that the in-vivo stability of the conjugates is comparable to or better than that of other steroidal systems designed for imaging purposes (*2*). The percent of unmetabolized conjugate in the uterus an hour after injection was also determined: for the ^{99m}Tc conjugate, 89%, and for the ^{186}Re conjugate, 60%. The radiochemical purity of the ^{186}Re conjugate in the uterine sample might be reduced because of air oxidation during sample work-up (*27*).

DISCUSSION

We have prepared ^{99}Tc-, ^{99m}Tc-, and ^{186}Re-labeled progestin conjugates **3–5** and measured their receptor binding in vitro and their tissue distribution and metabolic stability in vivo. While both the syn and anti diastereomers of the low specific activity ^{99}Tc and the unlabeled rhenium complexes (**3** and **6**, respectively) can be isolated (and the anti diastereomers separated) with the high specific activity complexes ^{99m}Tc (**4**) and ^{186}Re (**5**), only the more abundant syn diastereomers can be obtained (as an inseparable mixture). Both conjugates (**4** and **5**) have very high affinity for the progesterone receptor that exceeds that of progesterone, and even though the complexes are much more lipophilic than the steroids, they show pronounced specific binding in in vitro experiments.

The ^{99m}Tc and ^{186}Re conjugates displayed progesterone receptor-mediated uterine uptake in estrogen-primed immature rats. While the ^{99m}Tc conjugate displayed somewhat higher uterine uptake and longer uterine retention than the ^{186}Re conjugate, both were blocked by ORG2058 to an equivalent extent. The ^{186}Re conjugate did have a higher uterus/muscle ratio than the ^{99}Tc complex, but both show high non-target tissue uptake, most likely because of their high lipophilicity. As is the case with most steroid receptor ligands, the metabolic stability of both complexes in target tissue is good.

While the technetium and rhenium complexes have similar physical properties (*26,27*) (e.g., HPLC elution times), the rhenium complex appears to show somewhat higher nonspecific binding in vitro and lower target tissue uptake in vivo. It is possible that the technetium complex is less prone to increasing its coordination sphere to six coordinate, so that nucleophiles from non-target species do not associate with the metal. (There are studies that imply perrhenate is prone to increase its coordination sphere by associating with ligands (*26*), and although our system is an Re(V) species, the same phenomenon may be occurring.) An alternative explanation is that the rhenium complex, being more prone to oxidative degradation (*27*), appears in some assays (in vitro binding, in vivo uptake) to have higher nonspecific binding or uptake. Future studies are needed to resolve these issues.

These radiometal-labeled steroids are the first examples of a technetium- or rhenium-labeled small molecule to display very high affinity for a targeted receptor (*9,10*). Clearly, the biodistribution of these conjugates must be

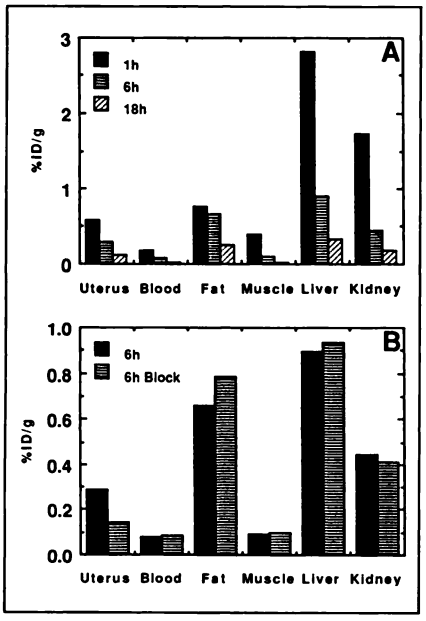


FIGURE 5. Comparison of the uptake of the ^{186}Re conjugate **5** in various rat tissues. (A) Uptake in target tissue (uterus) and various tissues at 1, 6, and 18 hr. (B) Uptake after 6 hr for rat coinjected with 18 μg ORG2058 to block the progesterone receptor and rats not coinjected.

improved, but this is a workable challenge since the chelate and linking groups are amenable to modification. It is hoped that ^{99m}Tc and ^{186}Re progestin conjugates with improved target uptake efficiency and selectivity will be good candidates for future studies aimed at the ultimate goal of obtaining images of progesterone receptor-positive breast tumors.

APPENDIX

Spectroscopic and microanalytical characterization of 11β-N_2S_2 ^{99}Tc-chelate, **3**.

Analysis for anti diastereomer **3anti1**: 1H-NMR (CD_2Cl_2, 300 MHz) δ7.240 (s, 4H, Ar-H), 5.720 (s, 1H, 4-CH), 4.453 (d-br, 1H, J = 7.26 Hz, 11-CH), 3.98–4.07 (m, 1H, NC*H*HCH$_2$N), 4.011 (d, 1H, J = 13.18 Hz, AmideNC*H*HC(CH$_3$)$_2$), 3.799–3.840 (m, 1H, NC*H*HCH$_2$N), 3.775 (d, 1H, J = 14.71 Hz, AmineNC*H*HC(CH$_3$)$_2$), 3.588 (ABq, 2H, Δδ = 0.244, J = 11.96 Hz, ArCH$_2$N), 3.514–3.615 (m, 1H, NC*H*HCH$_2$N), 3.278 (dd, 1H, J = 4.27, 12.07, NC*H*HCH$_2$N), 3.214 (d, 1H, J = 13.18 Hz, AmideNC*H*HC(CH$_3$)$_2$), 1.997 (s, 3H, C(C*H*$_3$)(CH$_3$), 1.874 (s, 3H, C≡C-CH$_3$), 1.783 (s, 3H, C(C*H*$_3$)(CH$_3$), 1.684 (s, 3H, C(C*H*$_3$)(CH$_3$), 1.434 (s, 3H, C(C*H*$_3$)(CH$_3$), 0.438 (s, 3H, 18-CH$_3$); IR (KBr) 1651 (3-ketone), 930 cm^{-1} (Re=O). MS (LRFAB) m/z (relative intensity) 763 (M$^+$+H+16, 2), 747 (M$^+$+H, 22), 613 (13), 515 (9), 474 (6); HRFAB Calcd for $C_{38}H_{51}O_3N_2S_2$^{99}Tc (+H): 747.2480, found 747.2463.

Analysis for anti diastereomer **3anti2**: ^{1}H-NMR (CD_2Cl_2, 300 MHz) δ7.240 (s, 4H, Ar-H), 5.718 (s, 1H, 4-CH), 4.447 (d-br, 1H J = 7.28 Hz, 11-CH), 3.99–4.07 (m, 1H, NC*H*HCH$_2$N), 3.998 (d, 1H, J = 13.31 Hz, AmideNC*H*HC(CH$_3$)$_2$), 3.780–3.830 (m, 1H, NC*H*HCH$_2$N), 3.768 (d, 1H, J = 14.54 Hz, AmineNC*H*HC(CH$_3$)$_2$), 3.568 (ABq, 2H, Δδ = 0.261, J = 11.99 Hz, ArCH$_2$N), 3.517–3.616 (m, 1H, NC*H*HCH$_2$N), 3.292 (dd, 1H, J = 4.37, 12.05, NC*H*HCH$_2$N), 3.209 (d, 1H, J = 13.31 Hz, AmideNCHHC(CH$_3$)$_2$), 1.998 (s, 3H, C(C*H*$_3$)(CH$_3$), 1.871 (s, 3H, C≡C-CH$_3$), 1.781 (s, 3H, C(C*H*$_3$)(CH$_3$), 1.680 (s, 3H, C(C*H*$_3$)(CH$_3$), 1.434 (s, 3H, C(C*H*$_3$)(CH$_3$), 0.433 (s, 3H, 18-CH$_3$); IR (KBr) 1651 (3-ketone), 928 cm^{-1} (Re=O). MS (LRFAB) m/z (relative intensity) 763 (M$^+$+H+16, 2), 747 (M$^+$+H, 22), 730 (6); HRFAB Calcd for $C_{38}H_{51}O_3N_2S_2$^{99}Tc (+H): 747.2480, found 747.2463.

Analysis for syn diastereomeric pair **3syn1,2**: ^{1}H-NMR (CD_2Cl_2, 300 MHz) δ7.337 (ABq, 4H, Δδ = 0.049, J = 7.945 Hz, Ar-H), 5.727 (s, 1H, 4-CH), 5.022 (ABq, 2H, Δδ = 0.354, J = 14.17 Hz, ArCH$_2$N), 4.579–4.588 (m, 1H, NC*H*HCH$_2$N), 4.486 (d-br, 1H, J = 7.04 Hz, 11-CH), 3.860 (s-br, 2H, AmideNC*H*$_2$C(CH$_3$)$_2$), 3.600 (d, 1H, J = 13.46 Hz, AmineNC*H*HC(CH$_3$)$_2$), 3.180–3.350 (m, 2H, NC*H*HC*H*HN), 1.922 (s, 3H, C(C*H*$_3$)(CH$_3$), 1.877 (s, 3H, C≡C-CH$_3$), 1.778 (s, 3H, C(C*H*$_3$)(CH$_3$), 1.529 (s, 3H, C(C*H*$_3$)(CH$_3$), 1.479 (s, 3H, C(C*H*$_3$)(CH$_3$), 0.460 (s, 3H, 18-CH$_3$); IR (KBr) 1651 (3-ketone), 920 cm^{-1} (Re=O). MS (LRFAB) m/z (relative intensity) 763 (M$^+$+16+H, 15), 747 (M$^+$+H, 23), 730 (10), 515 (6), 399 (9); HRFAB Calcd for $C_{38}H_{51}O_3N_2S_2$^{99}Tc (+H): 747.2480, found 747.2470. Anal. Calcd for $C_{38}H_{51}O_3N_2S_2$^{99}Tc: C, 61.10; H, 6.88; N, 3.75; S, 8.58. Found: C, 61.44; H, 7.23; N, 3.62; S, 8.37.

ACKNOWLEDGMENTS

We are grateful for support of this research through grants from the Department of Energy (DE FG02, 86ER60401 to J.A.K.; DE FG02 84ER60218 to M.J.W.; DE FG02 86ER60460 to A.D.). Certain steroids were kindly supplied by Dr. F. Zeelen of Organon and Dr. Teutsch of Roussel-Uclaf.

REFERENCES

1. Kiesewetter DO, Kilbourn MR, Landvatter SW, Heiman DF, Katzenellenbogen JA, Welch MJ. Preparation of four fluorine-18-labeled estrogens and their selective uptakes in target tissues of immature rats. *J Nucl Med* 1984; 25:1212–1221.
2. Pomper MG, VanBrocklin H, Thieme AM, et al. 11β-Methoxy-, 11β-ethyl-, and 17α-ethynyl-substituted 16α-fluoroestradiols: receptor-based imaging agents with enhanced uptake efficiency and selectivity. *J Med Chem* 1990;33:3143–3155.
3. Katzenellenbogen JA, Senderoff SG, McElvany KD, O'Brien HA, Jr, Welch MJ. [^{77}Br]-16α-Bromoestradiol-17β: a high specific-activity gamma-emitting tracer with uptake in rat uterus and induced mammary tumors. *J Nucl Med* 1981;22:42–47.
4. Katzenellenbogen JA, McElvany KD, Senderoff SG, Carlson KE, Landvatter SW, Welch MJ. 16α-[^{77}Br]-Bromo-11β-methoxyestradiol-17β. A gamma-emitting estrogen imaging agent with high uptake and retention by target organs. *J Nucl Med* 1982;23:411–419.
5. McElvany KD, Carlson KE, Welch MJ, Senderoff SG, Katzenellenbogen JA. In vivo comparison of 16α-[^{77}Br]-bromoestradiol-17β and 16α-[^{125}I]-iodoestradiol-17β. *J Nucl Med* 1982;23:420–424.
6. Hanson RN, Franke LA. Preparation and evaluation of 17α-[^{125}I]iodovinyl-11β-methoxyestradiol as a highly selective radioligand for tissues containing estrogen receptors: concise communication. *J Nucl Med* 1984;25: 998–1002.
7. Jagoda EM, Gibson RE, Goodgold H, et al. 17α-[I-125]Iodovinyl-11β-methoxyestradiol: in vivo and in vitro properties of a high-affinity estrogen-receptor radiopharmaceutical. *J Nucl Med* 1984;25:472–477.
8. Hochberg RB, Hoyte RM, Rosner W. E-17α-(2-[^{125}I]iodovinyl)-19-nortestosterone: the synthesis of a gamma-emitting ligand for the progesterone receptor. *Endocrinology* 1985;117:2550–2552.
9. Ballinger JR, Gulenchyn KY, Hassan MN. Technetium-99m-spiperone dithiocarbamate: a potential radiopharmaceutical for dopamine receptor imaging with SPECT. *Appl Radiat Isot* 1989;40:547–549.
10. Lever SZ, Wagner HM. The status and future of technetium-99m radiopharmaceuticals. In: Nicolini M, Bandoli G, Mazzi U, eds. *Technetium and rhenium in chemistry and nuclear medicine, volume 3*. New York: Raven Press; 1990:648–659.
11. DiZio, JP, Fiaschi R, Davison A, Katzenellenbogen JA. Progestin-rhenium complexes: Metal-labeled steroids with high receptor binding affinity, potential receptor-director agents for diagnostic imaging or therapy. *Bioconjugate Chem* 1991;2:353–366.
12. Brandes SJ, Katzenellenbogen JA. Fluorinated androgens and progestins: molecular probes for androgen and progesterone receptors with potential use in positron emission tomography. *Molec Pharmacol* 1987;32: 391–403.
13. Pinney KG, Carlson KE, Katzenellenbogen JA. [^{3}H]DU41165: a high affinity ligand and novel photoaffinity labeling reagent for the progesterone receptor. *J Steroid Biochem* 1990;35:179–189.
14. Katzenellenbogen JA, Johnson HJ, Myers HN. Photoaffinity labels for estrogen binding proteins of rat uterus. *Biochemistry* 1973;12:4085–4092.
15. Katzenellenbogen JA, Carlson KE, Johnson HJ, Myers HN. Estrogen photoaffinity labels II: reversible binding and covalent attachment of photosensitive hexestrol derivatives to the uterine estrogen receptor. *Biochemistry* 1977;16:1970–1976.
16. Minick DJ, Frenz JH, Patrick MA, Brent DA. A comprehensive method for determining hydrophobicity constants by reversed-phase high-performance liquid chromatography. *J Med Chem* 1988;31:1923–1933.
17. Scatchard G. The attractions of proteins for small molecules and ions. *Ann NY Acad Sci* 1949;51:660–672.
18. Mathias CJ, Welch MJ, Katzenellenbogen JA, et al. Characterization of the uptake of 16α-([^{18}F]fluoro)-17β-estradiol in DMBA-induced mammary tumors. *Nucl Med Biol* 1987;14:15–25.
19. Davison A, Orvig C, Trop HS, Sohn M, DePamphilis BV, Jones AG. Preparation of oxobis(dithiolato) complexes of technetium(V) and rhenium(V). *Inorg Chem* 1980;19:1988–1992.
20. Baidoo KE, Lever SZ. Evaluation of a diaminedithiol-based bifunctional chelate for labeling small molecules with ^{99m}Tc. In: Nicolini M, Bandoli G, Mazzi U, eds. *Technetium and rhenium in chemistry and nuclear medicine, volume 3*. New York: Raven Press; 1990:369–374.

21. Hiskey RG, Tomishige M, Igeta H. Sulfur-containing polypeptides. II. Selective removal of S-protective groups from some L-cysteinyl-L-cysteine derivatives. *J Org Chem* 1966;31:1188–1192.
22. Brenner D, Davison A, Lister-James J, Jones A. Synthesis and characterization of a series of isomeric oxotechnetium(V) diamido dithiolates. *Inorg Chem* 1984;23:3793–3797.
23. Mahmood A, Baidoo KE, Lever SZ. Stereoisomers of neutral oxotechnetium(V) and oxorhenium(V) complexes. In: Nicolini M, Bandoli G, Mazzi U, eds. *Technetium and rhenium in chemistry and nuclear medicine, volume 3.* New York: Raven Press; 1990:119–124.
24. Ojasoo T, Dore J-C, Gilbert J, Raynaud J-P. Binding of steroids to the progestin and glucocorticoid receptors analyzed by correspondence analysis. *J Med Chem* 1988;31:1160–1169.
25. Pomper MG, Katzenellenbogen JA, Welch MJ, Brodack JW, Mathias CJ. 21-[^{18}F]Fluoro-16α-ethyl-19-norprogesterone: synthesis and target tissue selective uptake of a progestin receptor based radiotracer for positron emisson tomography. *J Med Chem* 1988;31:1360–1363.
26. Deutsch E, Libson K, Vanderheyden J-L, Ketring AR, Maxon HR. The chemistry of rhenium and technetium as related to the use of isotopes of these elements in therapeutic and diagnostic nuclear medicine. *Nucl Med Biol* 1986;13:465–477.
27. Deutsch E, Libson L, Vanderheyden JL. The inorganic chemistry of technetium and rhenium as relevant to nuclear medicine. In: Nicolini M, Bandoli G, Mazzi U, eds. *Technetium and rhenium in chemistry and nuclear medicine, volume 3.* New York: Raven Press; 1990:13–22.
28. Katzenellenbogen JA, Heiman DF, Carlson KE, Lloyd JE. In vivo and in vitro steroid receptor assays in the design of estrogen radiopharmaceuticals. In: Eckelman WC, ed. *Receptor binding radiotracers, volume 1.* Boca Raton, FL: Chemical Rubber Co., 1982:93–126.
29. Keightley DD. The binding of progesterone, R-5020 and ORG-2058 to progesterone receptor. *Eur J Cancer* 1979;15:785–790.
30. Manz B, Grill H-J, Pollow K. Steroid side-chain modification and receptor affinity: binding of synthetic derivatives of corticoids to human spleen tumor and rat liver glucocorticoid receptors. *J Steroid Biochem* 1982;17:335–342.
31. Sheppard KE, Funder JW. Equivalent affinity of aldosterone and corticosterone for type I receptors in kidney and hippocampus: direct binding studies. *J Steroid Biochem* 1987;28:737–742.
32. Pomper MG. Fluorine-18 labeled estrogens, progestins and corticosteroids for receptor-based imaging of breast tumors and target areas of the brain. PhD thesis. University of Illinois, 1989.
33. Swinscow TDV. The t-tests. In: *Statistics at square one.* Bath, England: Mendip Press; 1980:33–42.

(continued from p. 544)

SELF-STUDY TEST

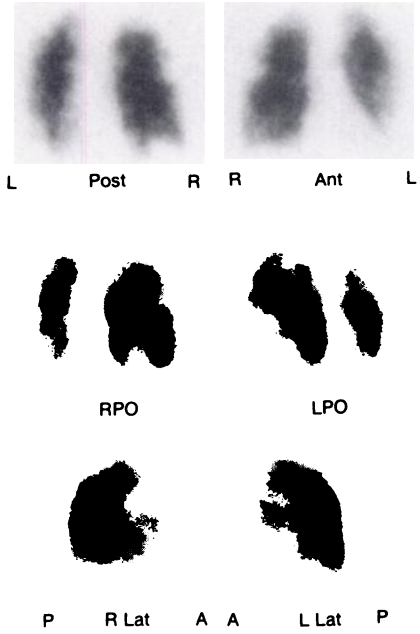


Figure 2C

3. A 45-yr-old white housewife with a 40 pack-year smoking history presents with progressive shortness of breath and an 8-pound weight loss. She denies febrile episodes, frequent respiratory infections, or excessive sputum production, but she has a dry cough. Her husband is bisexual. Clinical and laboratory examinations reveal cervical and inguinal lymphadenopathy and liver function abnormalities. Her ^{67}Ga scintigram is shown in Figure 3. Which *one* of the following is the most likely diagnosis?

A. bronchogenic carcinoma with lymph node metastases
B. Hodgkin's lymphoma
C. acquired immunodeficiency syndrome with *Pneumocystis carinii* pneumonia
D. sarcoidosis
E. hypersensitivity pneumonitis

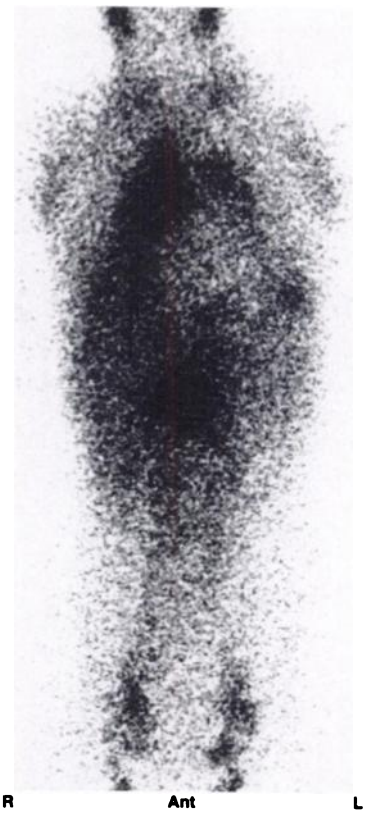


Figure 3

4. Which *one* of the following properties of a radioaerosol is *least* important in determining its rate of clearance from the lung?

A. solubility
B. lipophilicity
C. droplet size
D. pulmonary blood flow rate
E. alveolar–capillary membrane permeability

(continued on p. 580)